(i i) MOLECULE TYPE: DNA (genomic)

(v i) ORIGINAL SOURCE:
(A) ORGANISM: Mycobacterium tuberculosis

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CATAGGAGCT TCC

What is claimed is:

1. A method for concurrently generating a secondary amplification product and an amplification product in a Strand Displacement Amplification (SDA) reaction, wherein the SDA reaction comprises (i) a DNA polymerase having strand displacing activity and lacking 5'-3' exonuclease activity and (ii) a restriction endonuclease which nicks a hemimodified double stranded restriction endonuclease recognition site, the method comprising:

a) hybridizing a signal primer to a target sequence and hybridizing a first SDA amplification primer to the target sequence upstream of the signal primer;

b) extending the hybridized signal primer on the target sequence to produce a signal primer extension product and extending the hybridized first SDA amplification primer on the target sequence such that extension of the first SDA amplification primer displaces the signal primer extension product from the target sequence;

c) hybridizing a second SDA amplification primer to the signal primer extension product and extending the hybridized second SDA amplification primer on the signal primer extension product to produce a second SDA amplification primer extension product comprising a newly synthesized strand and double stranded hemimodified recognition site for the restriction endonuclease;

d) nicking the hemimodified recognition site and displacing the newly synthesized strand from the signal primer extension product using the DNA polymerase;

e) hybridizing the signal primer to the displaced newly synthesized strand and extending the signal primer such that a double stranded secondary amplification product is generated.

2. The method of claim 1 further comprising detecting the secondary amplification product by means of a chemical modification or special nucleotide sequence incorporated into the signal primer.

3. The method of claim 2 wherein the secondary amplification product is detected by means of an affinity ligand or reporter group incorporated into the signal primer.

4. The method of claim 2 wherein the secondary amplification product is detected by means of a nucleotide sequence incorporated into the signal primer, the nucleotide sequence comprising a recognition site for a double-stranded DNA binding protein.

5. The method of claim 2 wherein the secondary amplification product is detected by means of a nucleotide sequence incorporated into the signal primer, the nucleotide sequence comprising a restriction endonuclease recognition site.

6. The method of claim 5 wherein the secondary amplification product is detected by cleaving the restriction endonuclease recognition site with a restriction endonuclease to generate a cleavage product, separating the cleavage product on the basis of size and detecting the cleavage product.

7. The method of claim 6 wherein the cleavage product is separated by filtration.

8. A method for concurrently generating a secondary amplification product and an amplification product in a Strand Displacement Amplification (SDA) reaction, wherein the SDA reaction comprises (i) a DNA polymerase having strand displacing activity and lacking 5'–3' exonuclease activity and (ii) a restriction enzyme which nicks a hemi-modified double stranded restriction endonuclease recognition site, the method comprising:

a) hybridizing a first signal primer to a first strand of a double-stranded target sequence and hybridizing a first SDA amplification primer to the first strand of the target sequence upstream of the first signal primer;

b) extending the hybridized first signal primer on the first strand to produce a first extension product and extending the hybridized first SDA amplification primer on the first strand such that extension of the first SDA amplification primer displaces the first extension product from the target sequence;

c) hybridizing a second signal primer to the first extension product and hybridizing a second SDA amplification primer to the first extension product upstream of the second signal primer;

d) extending the hybridized second signal primer on the first extension product to produce a second SDA extension product and extending the hybridized second amplification primer on the first extension product such that extension of the second SDA amplification primer displaces the second extension product from the first extension product;

e) hybridizing the first signal primer to the displaced second extension product and extending the hybridized first signal primer on the second extension product such that a double stranded secondary amplification product is generated.

9. The method of claim 8 further comprising detecting the secondary amplification product by means of a reporter group incorporated into the first signal primer and a modification to facilitate capture of the secondary amplification product incorporated into the second signal primer.

10. The method of claim 8 further comprising the steps of:

a) hybridizing the second SDA signal primer to a second strand of the double stranded target sequence and hybridizing the second amplification primer to the second strand of the target sequence upstream of the second signal primer;

b) extending the hybridized second signal primer on the second strand to produce a third extension product and extending the hybridized second SDA amplification primer on the second SDA strand such that extension of the second amplification primer displaces the third extension product from the second strand of the target sequence;

c) hybridizing the first signal primer to the displaced third extension product and hybridizing the first SDA amplification primer to the displaced third extension product upstream of the first signal primer;

d) extending the hybridized first signal primer on the third extension product to produce a fourth extension product and extending the hybridized first SDA amplification primer on the third extension product such that extension of the first SDA amplification primer displaces the fourth extension product from the third extension product;

e) hybridizing the second signal primer to the displaced fourth extension product and extending the second signal primer on the fourth extension product such that a double stranded secondary amplification product is generated.

11. The method of claim 10 further comprising detecting the secondary amplification product by means of a chemical modification or special nucleotide sequence incorporated into the signal primer.

12. The method of claim 11 wherein the secondary amplification product is detected by means of an affinity ligand or reporter group incorporated into the signal primer.

13. The method of claim 11 wherein the secondary amplification product is detected by means of a nucleotide sequence incorporated into the signal primer, the nucleotide sequence comprising a recognition site for a double-stranded DNA binding protein.

14. The method of claim 11 wherein the secondary amplification product is detected by means of a nucleotide sequence incorporated into the signal primer, the nucleotide sequence comprising a restriction endonuclease recognition site.

15. The method of claim 14 wherein the secondary amplification product is detected by cleaving the restriction endonuclease recognition site with a restriction endonuclease to generate a cleavage product, separating the cleavage product on the basis of size and detecting the cleavage product.

16. The method of claim 15 wherein the cleavage product is separated by filtration.

17. The method of claim 2 wherein the secondary amplification products are detected in concurrently with amplification of the target sequence in real-time.

18. The method of claim 2 wherein the secondary amplification products are detected post-amplification.

19. The method of claim 9 wherein the secondary amplification products are detected in concurrently with amplification of the target sequence in real-time.

20. The method of claim 9 wherein the secondary amplification products are detected post-amplification.

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